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THE APPLICATION OF NORMAL SOLUTIONS TO BIOLOGICAL PROBLEMS.

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JUDGING from recent investigations into the effects of ions upon plant and animal life, one is led to conclude that there is much confusion in regard to the interpretation of certain standard chemical solutions. It is not to be doubted that solutions have a very important part in biological functions, and because of their importance it is deemed advisable to attempt to place this branch of an important subject upon a firmer basis.

Since solutions of molecular concentration have been adopted for comparison, in place of the older percentage concentration solutions, there seems to be a great tendency to misinterpretation and confusion. Solutions prepared on the percentage basis are not now considered scientifically accurate when used for purposes of comparison, while those molecularly equivalent may be compared with scientific accuracy. The molecular and the equivalent solutions are the only ones here discussed.

Much of the physiological work done with molecular solutions proves upon examination to be faulty because of a misconception of the meaning of *normal* solutions, *gram-equivalent* per liter solutions, and *gram-molecule* per liter solutions. There are no less than three interpretations of what a normal solution is. One takes it to be a *gram-molecule* per liter of *solution.* Another takes it to be a *gram-equivalent* per liter of *solution.* Still another regards it as a gram-molecule or a gram-equivalent per liter of *water.* Some use the symbol *n* (meaning in chemistry a normal solution) and say nothing about the method of preparation.

There are two probable causes for this confusion. The analytic chemists have differed in their use of solutions. (Allen, *Chem. News* **40**: 239; also *Analyst* **13**: 181, cited by Sutton **18**,[1] p. 28.) Some (a very few) German chemists use a gram-molecule per liter and call it a *normal* solution. By far the greater number of modern analytical chemists, however, use a *gram-equivalent* per liter of *solution* as a normal solution. The physical chemist uses both the above mentioned methods of preparation, but is always clear as to the meaning, and calls only the *gram-equivalent* per liter the *normal* solution. Those who use the chemical symbol *n* without explanation fall under some shadow of doubt arising from the above mentioned confusion. The work, however, may be faultless and the interpretation accurate. The theory of the separation, in aqueous solutions, of the molecules of compounds into ions is now a generally accepted one, and it is partly because of the actions of these ions, and of the dissociation of the molecule into ions, that there is great need of uniformity and of chemical accuracy in the preparation of solutions. Here are some definitions of *normal* solutions.

1. FRESENIUS (**2**, p. 687): "Solutions of such strength that 1000^{cc} contain an amount of acid or base equivalent to one gram of hydrogen are normal solutions, *e. g.*,

HCl,	mol. wt.	36.46,	wt. in	1000^{cc}	of solution	36.46
H_2SO_4,	"	98 ,	"	"	"	49
Na_2CO_3	"	106.08,	"	"	"	53.04"

Fresenius' chemical table (p. 846) is based on the above mentioned method of preparation.

2. SUTTON (**18**, p. 28): "Normal solutions are prepared so that one liter of solution at 16° C. shall contain the hydrogen equivalent of the active reagent in grams ($H = 1$)," *e. g.*:

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[1] See bibliography at end.

HCl	1	mol. wt. in	grams per	liter of	solution
H_2SO_4	$\frac{1}{2}$	"	"	"	"
Na_3PO_4	$\frac{1}{3}$	"	"	"	"
$KMnO_4$	$\frac{1}{5}$	"	"	"	"
$K_2Cr_2O_7$	$\frac{1}{6}$	"	"	"	"(Talbot **19**, p.65)

3. Talbot (**19**, p. 64): "A normal solution as defined by Mohr (Talbot **19**, pp. 64, 65) contains in one liter 'one equivalent of the active reagent in grams.' The equivalent in grams may be defined as 'that quantity of the active reagent which contains, replaces, unites with, or in any way, directly or indirectly, brings into reaction one gram of hydrogen.'"

Miller and Kilikani (**11**, p. 22), state regarding normality of solutions that "like volumes are equivalent to one another."

On the other side of the question, however, there are a few who use gram molecule per liter solutions and call them *normal* solutions. Of these, two perhaps are worth mentioning, Muter and Menschutkins, both cited by Sutton (**18**, p. 28).

In Ostwald's own work (**13**) he uses both gram-equivalent per liter and gram-molecule per liter of solution, but never confuses them. He uses the gram-molecule solutions for demonstrating molecular conductivity, and some of these tables are to be found in his Lehrbuch (**2**, pp. 722–772). Ostwald (**15**, p. 281) gives Kohlrausch's (**6**) table of equivalent quantities (not molecular quantities) for several solutions and enunciates the principle that the *equivalent* conductivity is a direct measure of the velocity of the migration of the ions. Ostwald (**14**, p. 284) uses normal solutions (with table) in regard to surface-tension of solutions, and on pp. 170–172 he uses *molecular* solutions with table of reference for molecular lowering of the vapor-pressure. There are only two instances of different tables used by Ostwald in his work. It will thus be seen that he makes use of both kinds of solutions according as they best suit his particular purpose.

Van't Hoff (**23**, p. 117), referring to Ostwald, uses the term "so-called molecular conductivity," and he uses freely the term normality as applied to solutions. Hittorf's table (Le Blanc **7**,

p. 78), is based on the *gram-equivalent* per liter solution, and Le Blanc (**7**, p. 79), uses the symbol λ to designate *equivalent* conductivity as distinguished from *molecular* conductivity, which is expressed by Ostwald as μ. Moreover, Valson's (**24**) law of the moduli (tables) is based upon gram-equivalent (not molecular) solutions, as well as almost all the analytical *chemical* tables now available.

It is seen that the physical chemist recognizes two kinds of standard solutions, *molecular* solutions and *equivalent* solutions, calling the latter *normal* solutions. The analytical chemist uses one kind of solution, the normal solution, *i. e.*, the gram-equivalent per liter solution.

The mistakes resulting from misinterpretation of *normal* solutions, and from a confusion of the terms *gram-equivalent* and *gram-molecule* per liter will be better understood if a few particular cases be cited. Kahlenberg and True (**5**, p. 85), speak thus: "Chemically equivalent quantities (*i. e.*, molecular quantities) of the different substances were not compared;" also (p. 91), "expressed in gram-molecules or gram-equivalents per liter;" showing that they regard a gram-molecule per liter as exactly the same as a gram-equivalent per liter, which, in the case of many of the substances referred to (*e. g.*, H_2SO_4), is not correct. What brings out more prominently the importance of the error is the fact that the solution of H_2SO_4 (p. 92) was a *normal* solution (purchased from the chemist), that is a gram-equivalent solution, and was thought to be a gram-molecule solution. Their results show that $\frac{1}{6400}$ gram-equivalent per liter of H_2SO_4 is as toxic as $\frac{1}{3200}$ gram-equivalent per liter of HCl. In other words, the experiments seem to prove that the solution of H_2SO_4 was what they *thought it to be*, though what *it was not*. Heald (**4**, p. 125), referring to Kahlenberg and True, states that "in these experiments the solutions were prepared according to gram-equivalents." On pages 119–123 of Kahlenberg and True we find written *gram-mol.* per liter.

Similar mistakes were made by True (**20**, p. 184), where he says, referring to H_2SO_4, "and since it splits off two hydrogen ions from every molecule, it would have, in chemically equivalent quantities, twice the number of H ions found in HCl, and would have its death-limit at one half the concentration of the monobasic acid." This shows clearly that this author regarded chemical equivalent and molecular solutions as the same.

Kahlenberg and True (**5**, p. 6), say: "In the second column is the concentration just allowing growth, expressed in fractions of a gram-equivalent per liter of water." In the column referred to, the limit for H_2SO_4 is $\frac{1}{6400}$, and for $KHSO_4$ is $\frac{1}{6400}$ in equivalent solutions. Since the toxicity of potassium and of SO_4 at this concentration may be neglected (p. 6), the hydrogen in the $KHSO_4$ is as toxic as *twice* as much hydrogen in the case of H_2SO_4, since there is in chemically equivalent quantities of H_2SO_4 and $KHSO_4$ *twice* as much hydrogen in H_2SO_4 as there is in the $KHSO_4$.

Another instance of the error arising from confusing the gram-equivalent per liter with the gram-molecule per liter is shown in the work of Kahlenberg and True (**5**, p. 109). They say, referring to Ostwald (**16**), "the most dilute solutions with which he worked contained one gram-equivalent in 1024 liters." Ostwald's tables in this reference are *not* made on the gram-equivalent per liter plan, but on the *gram-molecule* per liter plan, and he so states (p. 174). "Die folgende Spalte enthalt unter '*m*' den Wert der relativen molekularen Leitfähigkeit; der Wert von μ_∞, auf welchen derselbe bezogen ist, findet sich in der Ueberschrift der Tabelle angegeben." Also p. 171, "Wo μ_s die molekulare Leitfähigkeit bei der Verdünnung v (in Litern auf in Grammolekulargewicht), μ_∞ den Grenzwert derselben bei unendlicher Verdünnung und c eine Konstante bedeutet. Drückt man die molekulare Leitfähigkeit in Bruchtheilen ihres Grenzwertes aus, setzt also $m = \frac{\mu_v}{\mu_\infty}$."

Kahlenberg and True (**5**, p. 115) give in their tables 56, 57, 58, 59, "concentration gm.-equival. per liter," and refer to Ostwald (**16**, p. 380). Now Ostwald's tables (pp. 369–422) are made *not* on the plan gram-equivalent per liter but *gram-molecule* per liter. No comment is necessary. Again Kahlenberg and True (**5**, p. 116), state, "as maleic acid at the dilution 1024 is dissociated 98.2 per cent. and fumaric acid 78.5 per cent., we should expect the latter to be less poisonous than the former if the toxic action be due to the H ions alone." In the table to which they refer we find maleic acid *92.8 per cent.* at 1024 and fumaric acid 78.5 at *2048*. They state further, referring to the two acids mentioned, "we do not place much reliance on the results obtained from these two acids as it is questionable whether the substances were perfectly pure." The discrepancy above referred to suggests another reason for unreliability of results.

True and Hunkel (**22**, p. 326), while using gram-molecule per liter in their paper, made reference to Kahlenberg and True (**5**, p. 92), where the tables are written gram-equivalent per liter. The one kind of table, of course, cannot apply directly to the other.

In regard to the method of preparation of solutions by dissolving a gram-equivalent of the salt, or a gram-molecule of the salt, as the case may be, in a liter of *water*, there is something to be said. Those who have made errors in this way are Pfeffer (**17**, p. 146), Detmer and Moor (**1**, p. 326), Kahlenberg and True (**5**, pp. 85, 87), True (**20**, pp. 184, 185), True (**21**, pp. 410, 411), Heald (**4**, p. 133), Garry (**3**, p. 298), True and Hunkel (**22**, p. 326), and Ostwald (**14**, p. 190). Pfeffer, Detmer and Moor, and Garry called them *normal* solutions. Kahlenberg and True called them gram-molecule solutions and gram-equivalent solutions, apparently interchangeably, but meaning generally molecular solutions. Ostwald is using Tammann's table which states that the solutions are made by dissolving *n* molecular weights, in grams, of the salts in 1000 grams of water.[2]

The objection to the above solutions is not so much to the method of preparing them as to the application to them of chemical or physical tables prepared on a different basis; and to the error arising from their being called by names which mean something different. A solution prepared by dissolving one gram-equivalent in a liter of *water* is quite different in concentration (in the case of strong solutions especially) from that prepared by dissolving one gram-equivalent in a liter of *solution*. The difference is so apparent that explanation is unnecessary.

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[2] MACDOUGAL (**10**, p. 51) says: "A normal solution of ethyl alcohol is made by adding 46 grams of absolute alcohol to a liter of distilled water." This, to be in any sense accurate, should read (after the word alcohol) *to sufficient water to make a liter of solution.* MacDougal's statement produces an error of about 58^{cc} in 1000^{cc}.

The use of the chemical symbol *n* to represent solutions without some explanation as to the method of preparation of the solution, is likely to cause some doubt to be cast upon the work. One will naturally infer that those who use the symbol without explanation are as likely to err as those who use it with explanation, but in making explanation show an error of interpretation; though this inference might be unfair.

Garry (**3**, p. 298) states: "To designate chemically equivalent solutions, the chemist uses fractional parts of the so-called *normal* solutions, *i. e.*, the solution made by dissolving the equivalent gram-molecule in one liter of water." He uses the symbol N to designate his solutions, but if he prepared them after the method indicted above he is not warranted in using the symbol for normal solutions. Chemical tables showing degree of dissociation will, of course, not apply to solutions so made.

True (**20**, p. 186) uses the term *n*-1024 in reference to Ostwald's tables (**16**), but he is not warranted in using this term because Ostwald's tables just referred to are *not* based on *normal* solutions but are molecular solutions. Heald (**4**, p. 138) uses the term *N* and refers to the same page of *Zeitschrift* for degree of dissociation. These tables do not apply.

In the works of Loeb (**9**), D. J. Lingle (**8**), and A. Moore (**12**) the symbol *n* is used to designate the solutions, but no explanation is given, though in the context there are indications that the symbol *n* is properly applied.

The results of the errors and misconceptions just referred to may be much or little according as the points brought out are of little or of much consequence. At all events, there seems need of establishing this part of physiology upon a more secure and accurate basis. The analytical chemists and the physical chemists have done an enormous amount of investigation upon solutions, and they have established tables for various purposes. Their tables and results are convenient, clear, and ready for use; and the only way to make proper use of them is to make the solutions according to the chemist's standard.

Sutton (**18**, p. 28, footnote) gives a piece of excellent advice in the following statement: "Anyhow it is to be hoped that those who communicate processes to the chemical journals, or abstracts of foreign articles for publication, will take care to distinguish between the conflicting systems." If this advice is useful to the chemist, how much more useful should it be to those who are not chemists, but who in their work make use of tables of standard solutions made by the chemists.

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